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(54) COMPOSITIONS COMPRISING IL-2/hIL-2 AND METHODS FOR THEIR USE

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22. A method for enhancing the immune response of a human to a vaccine which method comprises administering hIL-2 to the human as part of the vaccination regime.

Claim

1. A composition for protecting an animal against a stress-induced syndrome, or for enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which composition comprises IL-2.

8. A method for protecting an animal against a stress-induced syndrome, or for enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which method comprises administering an ffective amount of IL-2 to the animal.

16. A composition for enhancing the immune response of a human to a vaccine which composition comprises hIL-2.

FORM 10

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Complete Specification for the invention entitled:

"COMPOSITION AND METHOD FOR TREATING ANIMALS"

The following statement is a full description of this invention, including the best method of performing it known to us

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COMPOSITION AND METHOD FOR TREATING ANIMALS

ABSTRACT

Methods and compositions for protecting an animal against stress-induced syndrome, or for enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which employ IL-2 as an active ingredient are described.

COMPOSITION AND METHOD FOR TREATING ANIMALS

Description

Technical Field

The invention relates to new uses for interleukin-2 (IL-2) in the field of veterinary medicine. More particularly, it concerns using IL-2 in controlling stress-related diseases in animals, or in enhancing the immune response of animals to vaccines, or in enhancing weight gain in animals.

Background

Stress-related Diseases

Livestock food animals, particularly cattle, are adversely affected by shipment and feedlot conditions, which involve stress from overcrowding, weaning, transport, sometimes severe weather, and, in general, a non-natural environment. One syndrome, commonly known as "shipping fever", is sometimes also designated "bovine respiratory disease syndrome", or BRDS. It is a complex of disease symptoms rather than a specific disease, and is characterized by immune suppression and propensity to succumb to infection by one or more viral, or bacterial pathogens.

Other animals are also subject to adverse reactions to stress. For example, pigs, while ordinarily not shipped in the manner of cattle, can suffer negative respiratory reactions to weaning or just to poor weather. Again, the symptomatology does not lend itself to xperimental models. No general treatment for stress-related disorders in livestock has been found. Sick animals are typically treated with antibiotics.

Recently several commercial entities have offered interferon preparati ns for treating shipping fever.

Vaccine Adjuvants

The use of vaccines to prevent diseases in farm livestock, sports animals and household pets is a common practice, and considerable effort has been, and is being, made to extend this practice to cover a more extensive array of diseases to which these animals are subject. For example, the use of rabies vaccine in animals is by now commonplace, and efforts are being made to obtain suitable vaccines to immunize animals against other diseases.

One problem that frequently is encountered in the course of active immunization is that the antigens used in the vaccine are not sufficiently immunogenic to raise the antibody titer to sufficient levels to provide protection against subsequent challenge or to maintain the potential for mounting these levels over extended time periods. Another problem is that the vaccine may be deficient in inducing cell-mediated immunity which is a primary immune defense against bacterial and viral infection. Notorious among such "weak" animal vaccines are those constituted from inactivated Haemophilus pleuropneumoniae (Hpp) (which is associated with respiratory disease in pigs).

In order to obtain a stronger humoral and/or cellular response, it is common to administer the vaccine in a formulation containing an adjuvant (immunopotentiator), a material which enhances the immune response of the patient to the vaccine. The most commonly used adjuvants for vaccines are oil preparations and alum. The mechanisms by which such adjuvants function are not understood, and whether or not a particular

adjuvant pr paration will be sufficiently effective in a given instanc is not predictable.

Animal Weight Gain

Extensive efforts have been made to encourage weight gain in animals being raised for food. Methods which are safe and effective tend also to be expensive, such as increasing the quality of the nutrition of the ubject animal. Therefore, attempts have been made to short circuit this direct approach by supplying hormones or other substances which cause metabolic changes in the animal to allow it to utilize its normal foodstuffs more efficiently. One example is the use of diethylstylbesterol (DES) to increase the weight of turkeys and cattle. While DES is apparently quite successful in effecting the weight increase, it has the serious disadvantage that residues remaining in the meat or other commercial product derived from the animal are sufficient to cause problems in the consumer population. Therefore, extensive regulation has been instituted to control the use of such additives.

Another example is the use of antibiotics in feed to reduce subclinical infection and disease and thereby improve the general health of the animal and its likelihood of gaining weight. The problem with such use of antibiotics is that they may give rise to resistant populations of organisms in the animal which may then be passed on to human consumers in the food chain. Control of infection by such resistant organisms with antibiotics would not be feasible.

Human IL-2 (hIL-2) Biological Activity

There is considerable background information available with respect to the biological activity of

hIL-2. hIL-2 can be obtained from the supernatant of concanavalin-A (ConA) stimulated spleen cells or, presently, using recombinant technology, and has several measurable activities in vitro. First, it is a T-cell growth factor as measured by, for example, thymidine uptake when hIL-2 is added to cultures of cytotoxic or helper T-cell lines. It is mitogenic with respect to adult thymocytes, and stimulates a cytotoxic cell response (e.g., lymphokine-activated-killer (LAK) cell). It has also been shown to replace helper T-cells in athymic murine spleen cell cultures (Watson, J., et al. Immunological Rev (1980) 51:257-278). Specifically, in the presence of IL-2 and antigen, specific T helper cells are generated which are able to contribute to antibody responses. Presumably this occurs because hIL-2 is involved in the antigen-dependent maturation of helper T-cells in these nude mouse spleen cultures.

IL-2 has also been shown to directly affect B cells in vitro. Both proliferation and IgM and IgG secretion are enhanced by IL-2 in populations of purified, activated B cells (Mingari, M.C., et al., Nature (1984) 312:641; Mittler, R., et al., J. Immunol. (1985) 134: 2393-2399; Muraguchi, A., et al., J. Exp. Med. (1985) 161:181-197).

How these in vitro activities translate into a specific in vivo mechanism for mounting an immune defense is not clear. However, with respect to such in vitro studies, cross-reactivity among species of various IL-2s has been studied. For example, Redelman, D., et al, J Immunol Meth (1983) 56:359-370) show that hIL-2 supports activated T lymphocytes derived from rabbit and mouse to approximately the same extent as they are support d by the endogenous forms of IL-2. Ruscetti, F.W., et al, Blood (1981) 57:379-393 were the first to

demonstrate the ability of hIL-2 to behave as a growth fact r, n t only f r human T-cells, but also p ripheral blocd lymphocytes or splenocytes from other primates, horse, guinea pig, cat, rat, and mouse. Carter, J., et al (Fed Proc (1985) 44:1290) disclose the ability of hIL-2 to enhance the development and maintenance of bovine cytotoxic lymphocytes in vitro.

Doyle, M.V., et al, J Bio Resp Mod (1985) 4: 96-109 reports in vitro lymphocyte proliferation studies that compared the activities of native hIL-2 and a recombinant form of hIL-2 on human and animal lymphocytes. The native IL-2 and recombinant IL-2 exhibited the same range of activity on animal cells.

Some in vivo data are also available. The activity of IL-2 in vivo has been shown to restore immunocompetence in nude mice in response to heterologous erythrocytes (Stötter, H., et al, Eur J Immunol (1980) 10: 719-722). There is information concerning cross-species reactivity, as well. Reed, S.G., et al, J Immunol (1984) 133:3333, disclosed the ability of hIL-2 to reconstitute spleen cell responses in mice infected with a parasitic protozoan, and Farrar, J.J., et al, Immunol Rev (1982) 63:158, showed that in vivo injection of hIL-2 stimulates the splenic T-cells in nude mice.

In summary, it is known that hIL-2 behaves in some manner in vivo to mediate a successful immune response, including a response to a specific antigen, and in vitro studies have shown that cross-species reactivity of hIL-2 is very diverse (prior in vivo cross-species studies have involved only murine subjects for hIL-2).

However, because the nature of IL-2 activity in vivo is not completely understood and because the mechanism of involvement of IL-2 in the immune response is

not understood, it is not, at this time, possible to predict its effect on particular diseases, other therapeutic or prophylactic regimens, or metabolism. Accordingly, there is no suggestion in the art that hIL-2 would successfully mitigate the incidence of stress-related syndromes in livestock, enhance the efficacy of vaccines, or enhance weight gain in animals.

Disclosure of the Invention

One aspect of the invention is a composition for protecting an animal against stress-induced syndromes, or for enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which composition comprises IL-2.

Another aspect is a method for protecting an animal against stress-induced syndromes, or enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which method comprises administering an effective amount of IL-2 to the animal.

When used as a vaccine adjuvant, hIL-2 is effective on humans as well as animals. Accordingly, another aspect of the invention is a composition for enhancing the immune response of a human to a vaccine which composition comprises hIL-2. In terms of a method, this aspect of the invention is a method for enhancing the immune response of a human to a vaccine which method comprises administering hIL-2 to the human as part of the vaccination regime.

Brief Description of the Drawings

Figure 1 shows the amino acid sequence of hIL-2.

Figures 2A and 2B are dose-response curves showing the results of the lymphocyte proliferation tests described in section C.1 of the examples, infra.

Figure 3 shows the effect of hIL-2 on blastogenesis of bovine and porcine T-lymphocytes.

Modes of Carrying Out the Invention

A. Definitions

As used herein, "hIL-2" refers to a polypeptide exhibiting the spectrum of activities characterizing this protein. Specifically, the protein must be capable of stimulating the proliferation of hIL-2 dependent cytolytic and helper T cell lines, as set forth in the standard assays of Gillis, S. et al., J Immunol (1978) 120:2027-2032 and of Watson, J. J Exp Med (1979) 150:1510-1519. The amino acid sequence of native hIL-2 is shown in Figure 1. This primary amino acid sequence may be obtained as the native protein from natural sources or may be recombinantly derived. Other primary sequences of modest modification including deletion, addition, substitution or alterations of the amino acids of the sequence shown, which do not result in serious impairment of activity are, of course, included in the definition. For example, it is established that replacement of the cysteine at position 125 with a neutral amino acid results in a mutein of superior stability and satisfactory reactivity. (See U.S. Patent No. 4,518,584; Doyle, M.V., et al, supra.)

In addition, hIL-2, like any other protein, may exist in neutral or in salt form, and may contain associated nonprotein moieties in the nature of glycosylation, phosphorylation, or acetylation. These modifications, too, are included in the definition so long as biological activity is not destroyed thereby.

The word "IL-2" herein also includes bovine IL-2 as described by Cerretti et al., P.N.A.S., 83: 3223-3227 (1986).

As used herein the term "stress-induced syndrome" refers to a state of immunosuppression in which

an animal has a propensity to succumb to infection by on or more bacterial or viral pathogens, lose weight, or exhibit general ill health.

"Shipping fever" or "bovine respiratory disease syndrome" (BRDS) is defined as negative symptomatology including depression, immunosuppression, weight loss, respiratory problems, viral or bacterial infection, and general ill health and death which are associated with the transportation of cattle to, and the maintenance of cattle on, feedlots. The disease is defined in terms of epidemiology rather than in terms of a model which describes the course of an infection or specific set of metabolic parameters. The criterion for effectiveness against this disease is the maintenance of healthy animals faced with the specific conditions associated with shipping stress and feedlot maintenance. Certain parameters of the disease are recognized. It is characterized by an abrupt onset, usually within two weeks of stress, and the symptoms may include dyspnea, cough, ocular and nasal discharge, inappetance and rapid weight loss, fever, increased lung sounds, and general depression. Various bacteria and viral cultures have been isolated from affected animals, including Pasteurella spp, Haemophilus spp, infectious bovine rhinotracheitis, parainfluenzavirus, and bovine respiratory syncytial virus. The disease typically affects 40-50% of exposed animals and the resulting deaths are typically 2-5% of the exposed population.

As used herein, the term "adjuvant" has its conventional meaning, i.e., the ability to enhance the immune response to a particular antigen. Such ability is manifested by a significant increase in immune-mediated protection. Enhancement of humoral immunity is typically manifested by a significant

increase (usually > 10%) in the titer of antibody raised to th antigen.

General Method

The formulations of the invention are most conveniently administered by intramuscular injections or as sustained release compositions although other methods of administration are possible. Specific formulations to prevent hydrolysis during digestion would be necessitated for oral formulation, and intravenous injections are generally uneconomic due to the skill level and care required in the administration. Therefore, formulations suitable for intramuscular injection. especially sustained release formulations, are preferred.

Standard formulations are either liquid injectables or solids which can be taken up in suitable liquids as suspensions or solutions for injection. Suitable excipients are, for example, water, saline, dextrose, glycerol, and ethanol. Nontoxic auxiliary substances, such as wetting agents, buffers, or emulsifiers may also be added. One specific useful formulation contains an effective amount of detergent, such as 0.1% sodium dodecyl sulfate (SDS), to effect solubility and bacteriostasis.

A variety of techniques are known in the art to effect long-term stability and slow release. For example, stability and half-life of hIL-2 are enhanced by coupling it to a hydrophilic polymer such as polyethylene glycol (PEG). This PEG-hIL-2 complex, called "PEGylated" hIL-2, is particularly useful for administering a single sustained action dose of hIL-2.

Sustained and continuous release formulations are of considerable variety, as is understood by those skilled in the art. An exemplary composition for sus-

tained releas parenteral administration is an injectable microcapsule formulation that with a single injection will deliver recombinant hIL-2 or soluble forms of hIL-2, such as PEGylated hIL-2, at a controlled rate of about 10^3 to 10^5 units/kg/day (pure hIL-2 has a specific activity of about 3-6 x 10^6 u/mg). The microcapsule formulation is a free-flowing powder consisting of spherical particles 20 to 100 μm in diameter that can be injected intramuscularly or subcutaneously with a conventional hypodermic needle, and the microcapsules consist of 0.5 to 5% hIL-2 encapsulated in poly(DL-lactide-co-glycolide) (DL-PLG) excipient, a biodegradable, biocompatible polyester. Alternative standard formulations for sustained release are also usable.

When used as a vaccine adjuvant, the hIL-2 will normally be administered separately from the vaccine, although it may, in some instances, especially in sustained or continuous release forms, be administered in combination with the vaccine. When hIL-2 is combined with the vaccine, the composition administered contains an immunogen that is effective in eliciting a specific response to a given pathogen or antigen, a pharmaceutically acceptable vaccine carrier and an immunopotentiating amount of hIL-2. A preferred regimen is to administer the hIL-2 continuously until 5 to 30 days, preferably 5 to 14 days, post-vaccination at levels above about 10^3 and below about 10^6 units/ kg/day. The term "continuously" is intended to denote true continuous administration, such as is achieved via a sustained release dosage form as well as a multiplicity of intermittent administrations of hIL-2 (or enhanced half-life forms of hIL-2 such as PEGylated hIL-2) that provide a

pharmacokinetic pattern that mimics that achieved by true continuous administration. Data generated to date using daily intramuscular injections indicate a preferred dose is 10^4 to 10^5 units/kg/day. The vaccine will normally be administered per manufacturer's instructions. Other adjuvants may be administered either with the vaccine or together with the hIL-2.

The hIL-2 will typically be used to enhance the protection afforded by animal or human vaccines that are considered "weak" (i.e., provide diminished protection in terms of level, extent, and/or duration). Examples of such vaccines are bacterins such as Bordetella bacterin, Escherichia coli bacterins, Haemophilus bacterins, Leptospirosis vaccines, Moraxella bovis bacterin, Pasteurella bacterin and Vibrio fetus bacterin and attenuated live or killed virus products such as bovine respiratory disease vaccine (infectious bovine rhinotracheitis, parainfluenza, respiratory syncytial virus), bovine virus diarrhea vaccine, equine influenza vaccine, feline leukemia vaccine, feline respiratory disease vaccine (rhinotracheitis-calici-pneumonitis viruses), canine parvovirus vaccine, transmissible gastroenteritis vaccine, and pseudorabies vaccine.

The regime of hIL-2 administration for protecting animals against shipping fever will depend on the conditions of shipment and the feedlot. It is preferred that administration be continuous and be begun prior to shipment or at least as early as arrival on the feedlot and be continued over a period of, for example, 14-30 or more days. Again, the term "continuous" is intended to denote true continuous administration, such as is achieved via a sustained release dosage form as well as a multiplicity of intermittent administrations of

hIL-2 (or enhanced half-life forms of hIL-2 such as PEGylated hIL-2) that provide a pharmacokinetic pattern that mimics that achieved by true continuous administration. Daily doses in the range of above about 10^3 and below about 10^6 units/kg/day, preferably about 10^4 to 10^5 units/kg/day, are generally used. In cattle, doses above about 10^6 units/kg/day began to cause undesirable side effects.

For other livestock stress-induced or respiratory distress syndromes, the regime and amounts administered will depend on the nature and size of the animal (e.g., pig, goat, sheep, etc.) and on the severity of the symptoms. It is likely, however, that the effective dose for such syndromes will be in the same (on a unit weight basis) range as that used for shipping fever.

The hIL-2 may be administered by itself or as a supplement to vaccines used to protect against stress-related diseases.

When used to enhance weight gain, the IL-2 is typically administered for prolonged periods (e.g., throughout the feeding schedule for the animals). The IL-2 dose for this use will normally be in the range of 10^3 to 10^5 units/kg/day.

C. Examples

The following examples are intended to further support or illustrate but not to limit the invention.

C.1. In Vitro Activity

In vitro activity with respect to bovine and porcine peripheral blood mononuclear cells (PBMC) has been shown for recombinant hIL-2 (Fong, Susan, et al. Vet Immunol and Immunopathol (1986) 11:91-100). The hIL-2 used in this work is designated des-alanyl-

rIL-2_{ser125}, lacks an initial alanine, and has a serine rather than a cysteine at position 125. It was shown to be mitogenic for unactivated bovine and porcine PBMC, and to be able to maintain the long-term growth of ConA-activated PBMC from both species. Figures 2A and 2B are curves showing the dose-response of ConA-activated bovine (2A) and porcine (2B) PBMC to des-alanyl-rIL-2_{ser125}. Also, bovine and porcine PBMC preincubated with des-alanyl-rIL-2_{ser125} for 1-5 days showed enhanced cytotoxicity against tumor cell targets.

In addition, Stott, J.L., et al (in press) have shown that bovine and porcine peripheral blood lymphocytes were responsive to human recombinant IL-2 in lymphocyte blastogenesis assays. Blastogenesis was determined by incorporation of ^{3}H-thymidine (18 hr pulse) in 4-day lymphocyte cultures, and the results expressed as the $\log_{10}$ of the geometric mean ($G_{\bar{x}}$) of disintegrations per minute (DPM)/culture and plotted by nonlinear regression analysis as shown in Figure 3. Mitogen dilution and concentration of hIL-2 in units are shown on the X-axis. These results show that the effect of hIL-2 on bovine and porcine cells is comparable to that shown by the plant lectins PHA and ConA, which are known to stimulate blastogenesis.

C.2. Potentiation of Cell-Mediated Immunity

Since respiratory diseases are predominantly controlled by the cellular (T-cell) immune system, the ability of hIL-2 to boost the cellular immune response in livestock is indicative of its effectiveness against these symptomologies. In vivo injections of recombinant hIL-2 produced elevated levels of lymphocyte blastogenesis in the blood of calves.

Specifically, eight calves weighing 135-225 kg (3-5 months old) were randomly sorted into 4 groups of 2 each which received weekly injections for one month as follows: Groups 1, 2, and 3 received 10^4, 10^5, and 10^6 units/kg, respectively, intramuscularly; group 4 received only excipient. The animals were assessed for lymphocyte stimulation. The results show that resting lymphocyte activity was elevated by the recombinant hIL-2 treatment as determined by blastogenesis assays performed prior to each inoculation over the period in calves receiving 10^5 and 10^6 units/kg only. For calves receiving 10^5 units/kg, lymphocyte activity returned to normal within two weeks following the last IL-2 administration; 10^6 units/kg-injected calves remained elevated at that time.

C.3. Treatment of Shipping Fever

Two hundred heifers were purchased from several different sources in Tennessee and transported to a research feedlot in Colorado. The average weight of the animals was approximately 180 kg. The animals were segregated randomly (weight and breed) into four groups, designated I through IV.

Recombinant hIL-2 (des-alanyl-rIL-2_{ser125}) was formulated in 0.05% SDS and administered intramuscularly to the animals upon entry to the feedlot. All animals were treated daily, five times per week, for two weeks. The dose protocols for the four groups were as follows.

Group	IL-2 Dose (u/kg/day)
I	2×10^4 (high dose)
II	2×10^3 (mid dose)
III	2×10^2 (low dose)
IV	control (diluent)

The animals did not receive standard BRDS-related vaccination. They were, by chance, subjected to severe snow and cold weather during their first days on the feedlot, and accordingly, were placed on silage feed early on. The health of the animals was observed on a daily basis by personnel blind to experimental treatment. The animals were weighed at regular intervals. Table 1 reports the results of the treatment as of day 21.

Table 1

Mortality

	Number Dead / Total	
Control	21 / 50	
Low Dose	20 / 50	p = 0.839
Mid Dose	26 / 50	p = 0.316
High Dose	14 / 50	p = 0.142

Incidence of Disease

	Number Sick or Dead / Total	
Control	43 / 50	
Low Dose	42 / 50	p = 0.779
Mid Dose	43 / 50	p = 1.000
High Dose	38 / 50	p = 0.202

Severity of Disease

Average Daily Severity Score of Group
(Score 0 - 3; Death = 4)

Control	1.76	
Low Dose	1.79	p = 0.950
Mid Dose	1.93	p = 0.395
High Dose	1.38	p = 0.052

Morbidity and mortality rates during the study were higher than expected. As reported some groups showed 85% morbidity and 50% mortality. Sickness was observed as early as two days into the study. Several factors may have been responsible for the extreme severity of BRDS seen in this study: the severe snow and cold weather; the animals were 'light-weight' (400 lbs avg) and 'thin-skinned' (from Tennessee); groups had been 'put-together' from several sources (thus, they were not 'fresh' and many had seen several salebarns prior to shipping to Colorado); and the animals were placed on silage feed early on, and may have been eating poorly.

In the clinical judgement of the personnel observing the health of the animals, the high-dose IL-2 group consistently "looked better". This is supported by the data in Table 1 in which the high-dose IL-2 group showed a consistent trend towards decreased mortality; decreased incidence of disease; and decreased severity of disease.

In all cases, the high-dose group performed better than the control group. Although the statistical significance of these differences (p-value), is marginal (using the strict definition of $p < 0.05$), all results are consistent.

Additional measures not presented in Table 1 also supported the trend toward efficacy in the highdose IL-2 group. For instance, animals in the high-dose group which died, did so later in the study than did control animals.

As of day 21, there were no differences in the average weight of surviving animals. There were, however, significant differences in the total pay-weight per group, since more animals survived in the high-dose group.

C.4. Effectiveness as an Adjuvant in Porcine Vaccine

Recombinant hIL-2 was shown to enhance the efficacy of an inactivated Hpp bacterin using 12 feeder pigs divided into 6 groups of 2 pigs each. Group 1 was an hIL-2 adjuvant control; group 2 was a bacterin control; group 3 received 10^3 units hIL-2/kg as a single injection on days 0 and 21; group 4 received 5 daily injections of 10^3 units/kg each following each vaccination; group 5 received 1 injection of 10^5 units/kg on days 0 and 21; and group 6 received 5 daily injections of 10^5 units/kg each following each vaccination.

The pigs in groups 2-6 were administered formalin-inactivated Hpp emulsified in an oil adjuvant intramuscularly in the neck muscles on days 0 and 21. The pigs in all groups were challenged on day 41 intranasally with serotype 1 Hpp and were killed on day 71 and autopsied. Lung area affected was determined visually with particular attention given to lung lesions. The pigs were weighed periodically during the 71 days -- with weight gain being an indication of general state of health. The results were as follows:

Group	Autopsy (% Lung Area Affected)	Average Rate of Body Weight Gain (kg/day) Days 0-41	Days 41-71
1	24.57%	0.69	0.30
2	6.100%	0.69	*
3	25.58%	0.62	0.27
4	12.12%	0.55	0.86
5	13.19%	0.62	0.39
6	0.0%	0.58	0.88

*pig died 5 days after challenge

As shown by these results the groups that received daily post-vaccination injections of hIL-2 (groups 4 and 6) exhibited substantially higher weight gain post-challenge than did the groups treated otherwise. These results also show a significant reduction of lung pathology in groups 4 and 6, indicating increased protection against challenge provided by the daily administration of hIL-2 in the vaccination regimen. All animals showed high antibody titers against Hpp after challenge.

A second study was carried out to confirm the ability of hIL-2 (des-alanyl-rIL-2_{ser125}) to act as an adjuvant to Hpp vaccination. The protocol for the second study was similar to that of the first study described above: animals were vaccinated with/without hIL-2 treatment and subsequently challenged with virulent Hpp. Principal measures of efficacy included clinical signs following infection, weight gain following infection, and the extent of lung involvement at necropsy. The results of this second study are tabulated below.

Group (N)	Ave. Percent Lung Affected At Necropsy	Ave. Daily Weight Gain (kg/day) Pre-Challenge	Post-Challenge
Control (2)	34	0.37	0.31
Hpp Alone (3)	19	0.34	0.62
Hpp + IL-2 (3) 10^5 u/kg daily	01	0.29	0.84
Hpp + IL-2 (2) 10^4 u/kg daily	00	0.43	0.75
No Challenge (2)	--	0.36	0.83

The data from the second study confirm the efficacy observed at 10^5 u/kg/day hIL-2 in the first study and extend these findings to the lower dose of 10^4 u/kg/day. Protection at an IL-2 dose of 10^4 u/kg/day was comparable to that observed at the higher dose.

C.5. Effectiveness as an Adjuvant in Dogs

Dogs were injected with keyhole limpet hemocyanin (KLH) at the time of initial hIL-2 treatment and 7 days subsequently at the start of 5 days of (daily) hIL-2 treatment. Enzyme-linked immunoabsorbent assays (ELISAs) were performed on sera taken from the dogs to measure antibody response to KLH. A significant, dose-dependent increase in IgG antibody against KLH was observed in the IL-2 treated dogs. The increase was specific to the KLH immunogen used.

C.6. Effectiveness in Increasing Weight Gain

The effect of recombinant hIL-2 administration, at various doses and schedules, on the weight gain of young pigs being raised in a normal environment is shown by the following tests.

Forty-eight weaned pigs, 15-20 kg, in normal health are divided into eight groups of six each. hIL-2 or control c mpositions are administered intramuscularly to the pigs as follows.

Group	Dose	Regimen
1	Low Dose (10^3 U/kg)	once weekly for four weeks
2	Mid Dose (10^4 U/kg)	once weekly for four weeks
3	High Dose (10^5 U/kg)	once weekly for four weeks
4	Low Dose (2×10^2 U/kg)	daily for five days in each of four weeks
5	Mid Dose (2×10^3 U/kg)	daily for five days in each of four weeks
6	High Dose (2×10^4 U/kg)	daily for five days in each of four weeks
7	Excipient control at high dose level	daily for five days in each of four weeks
8	Saline control	once weekly for four weeks

Individual weights at time 0 and weekly through 6-8 weeks are measured. Feed consumption and clinical impressions of illness are also recorded. Animals receiving hIL-2 have a significantly larger weight gain and are generally healthier than the animals of the control group.

~~CLAIMS~~

The claims defining the invention are as follows:

1. A composition for protecting an animal against a stress-induced syndrome, or for enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which composition comprises IL-2.

2. The composition of claim 1 wherein the IL-2 is hIL-2.

3. The composition of claim 2 wherein the hIL-2 is a water-soluble form of hIL-2.

4. The composition of claim 3 wherein the hIL-2 is PEGylated hIL-2.

5. The composition of claim 2, 3, or 4 wherein the hIL-2 is des-alanyl-rIL-2$_{ser125}$.

6. The composition of claim 1, 2, 3, 4, or 5 wherein the IL-2 is in the form of a continuous release formulation.

7. The composition of claim 1, 2, 3, 4, or 5 wherein the IL-2 is in the form of a single sustained action formulation.

8. A method for protecting an animal against a stress-induced syndrome, or for enhancing the immune response of an animal to a vaccine, or enhancing weight gain in an animal, which method comprises administering an effective amount of IL-2 to the animal.

9. The method of claim 8 wherein the IL-2 is hIL-2.

10. The method of claim 9 wherein the hIL-2 is a water-soluble form of hIL-2.

11. The method of claim 10 wherein the hIL-2 is PEGylated hIL-2.

12. The method of claim 9, 10, or 11 wherein the hIL-2 is des-alanyl-rIL-2_{ser125}.

13. The method of claim 8, 9, 10, 11 or 12 wherein the IL-2 is in the form of a continuous release formulation.

14. The method of claim 8, 9, 10, 11 or 12 wherein the IL-2 is in the form of a single sustained action formulation.

15. The method of claim 13 wherein the IL-2 is administered continuously at a rate above 10^3 and below 10^6 units/kg/day.

16. A composition for enhancing the immune response of a human to a vaccine which composition comprises hIL-2.

17. The composition of claim 16 wherein the hIL-2 is a water-soluble form of hIL-2.

18. The composition of claim 17 wherein the hIL-2 is PEGylated hIL-2.

19. The composition of claim 16, 17, or 18 wherein the hIL-2 is des-alanyl-rIL-2_{ser125}.

20. The composition of claim 16, 17, 18 or 19 wherein the IL-2 is in the form of a continuous release formulation.

21. The composition of claim 16, 17, 18 or 19 wherein the IL-2 is in the form of a single sustained action formulation.

22. A method for enhancing the immune response of a human to a vaccine which method comprises administering hIL-2 to the human as part of the vaccination regime.

23. The method of claim 22 wherein the hIL-2 is a water-soluble form of hIL-2.

24. The method of claim 23 wherein the hIL-2 is PEGylated hIL-2.

25. The method of claim 22, 23, or 24 wherein the hIL-2 is des-alanyl-rIL-2_{ser125}.

26. The method of claim 22, 23, 24, or 25 wherein the IL-2 is in the form of a continuous release formulation.

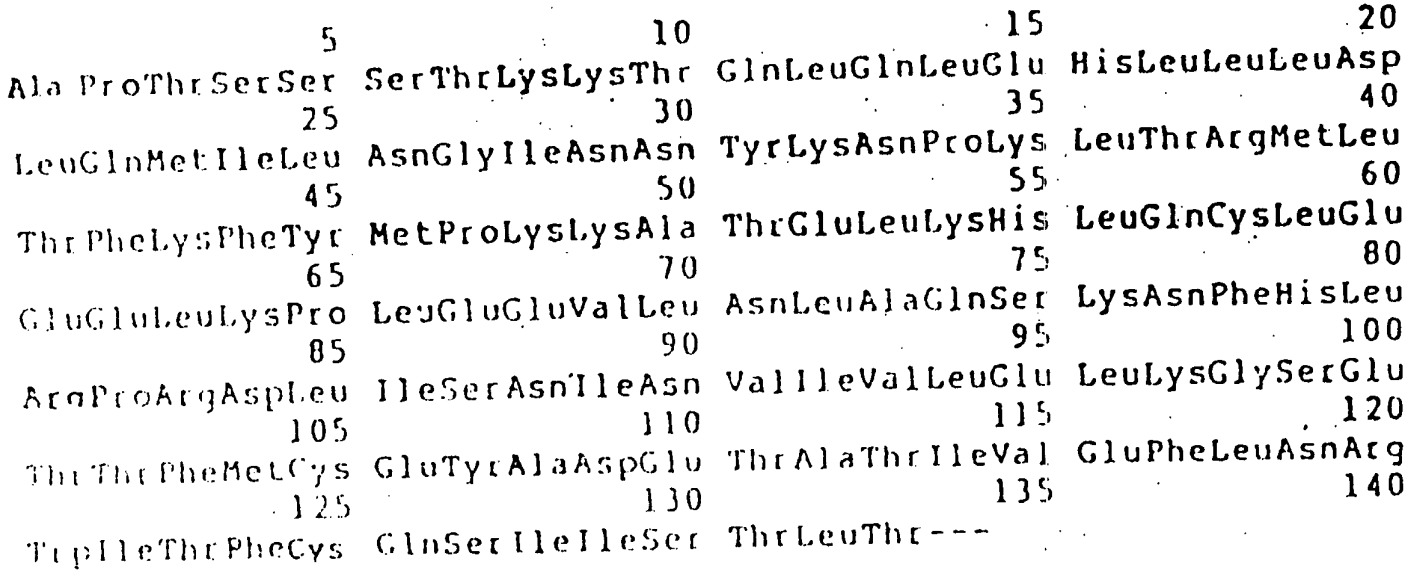
27. The method of claim 22, 23, 24, or 25 wherein the IL-2 is in the form of a single sustained action formulation.

DATED this EIGHTEENTH day of [illegible]

CETUS CORPORATION

Patent Attorneys for the [illegible]

HIRSCH & FERGUSON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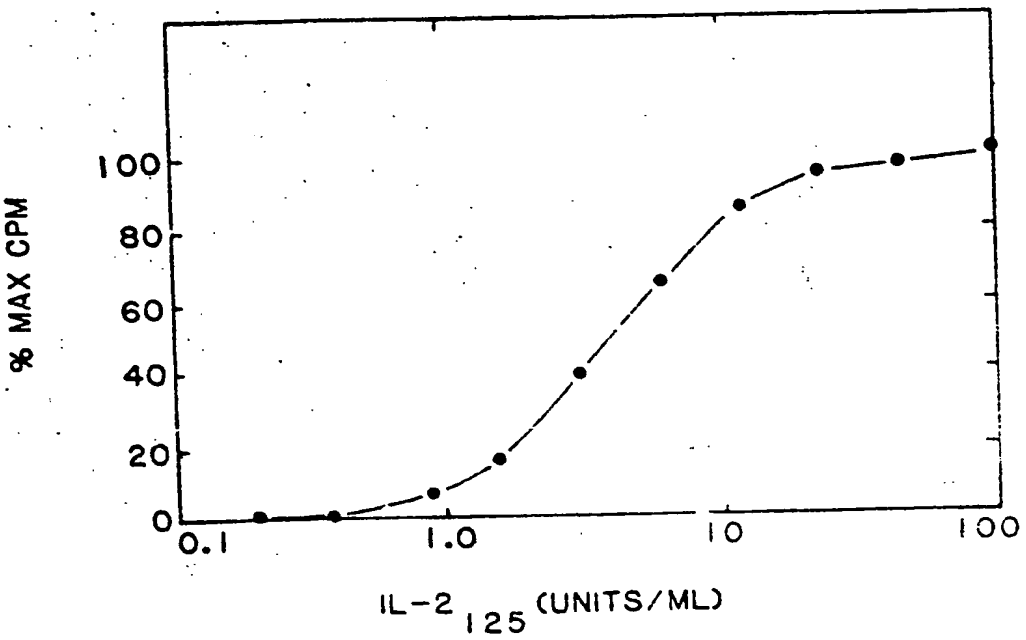
45 50 55 60
ThrPheLysPheTyr MetProLysLysAla ThrGluLeuLysHis LeuGlnCysLeuGlu
65 70 75 80
GluGluLeuLysPro LeuGluGluValLeu AsnLeuAlaGlnSer LysAsnPheHisLeu
85 90 95 100
ArgProArgAspLeu IleSerAsnIleAsn ValIleValLeuGlu LeuLysGlySerGlu
105 110 115 120
ThrThrPheMetCys GluTyrAlaAspGlu ThrAlaThrIleVal GluPheLeuAsnArg
125 130 135 140
TrpIleThrPheCys GlnSerIleIleSer ThrLeuThr---

FIG. 1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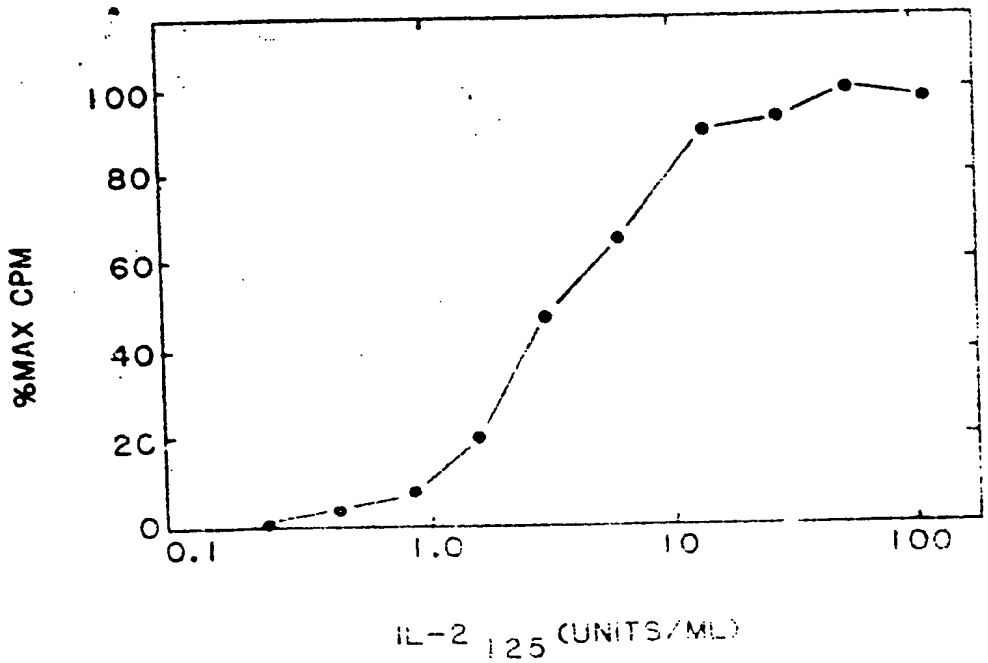
100
80
60
40
20
0
0.1
1.0
10
100
IL-2 125 (UNITS/ML)

FIG. 2A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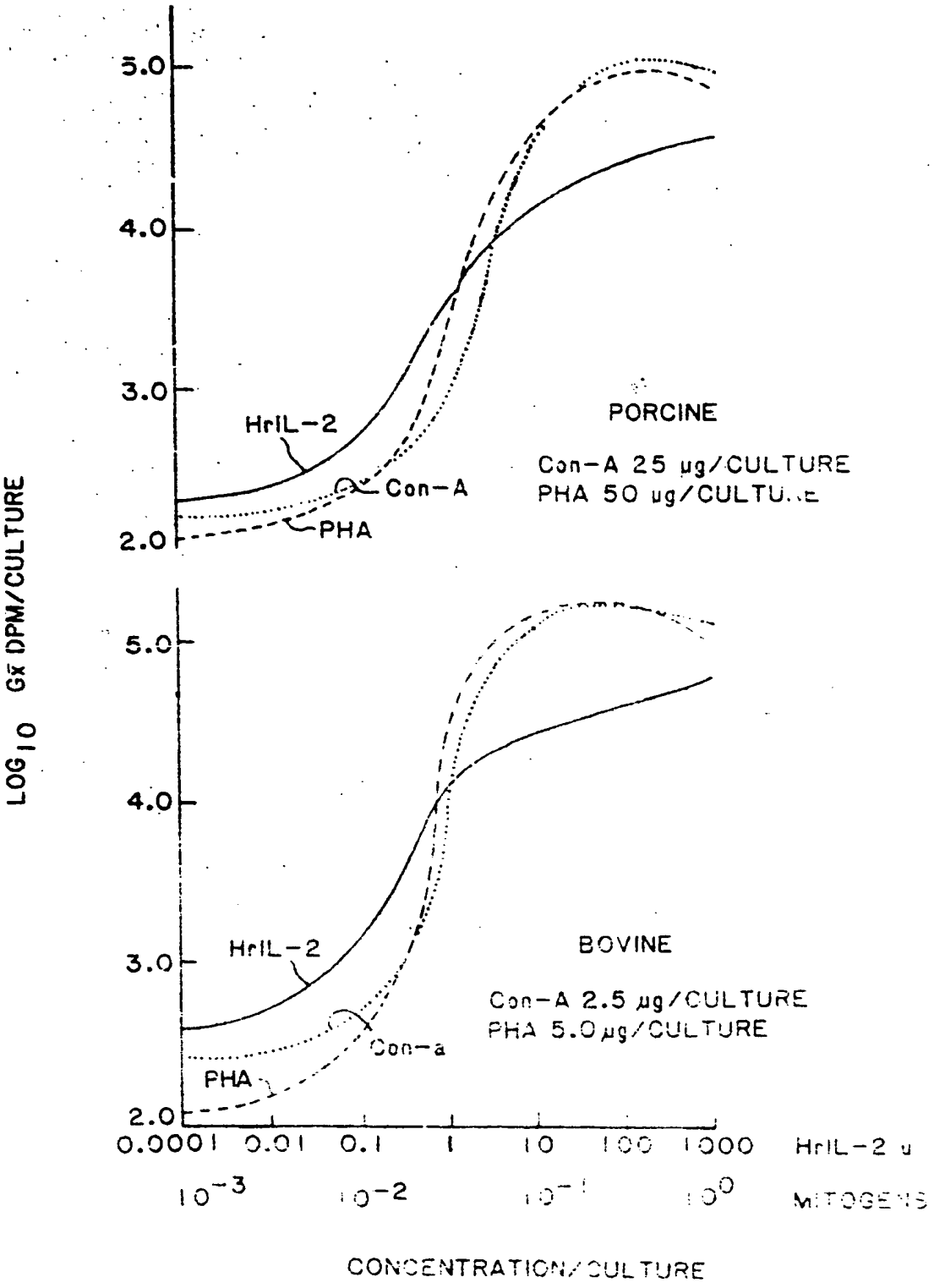
80
60
40
20
0
0.1
1.0
10
100
IL-2 125 (UNITS/ML)

FIG. 2B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4.0
3.0
2.0
HrIL-2
Con-A
PHA
PORCINE
Con-A 25 µg/CULTURE
PHA 50 µg/CULTURE
5.0
4.0
3.0
2.0
HrIL-2
Con-a
PHA
BOVINE
Con-A 2.5 µg/CULTURE
PHA 5.0 µg/CULTURE
0.0001
0.01
0.1
1
10
100
1000
HrIL-2 u
10-3
10-2
10-1
100
MITOGENS
CONCENTRATION/CULTURE

FIG. 3